THE ORIGIN OF THE GERM CELLS IN THE LAKE LAMPREY (*PETROMYZON MARINUS UNICOLOR*).

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Introduction.

Among the numerous articles concerning the sex cells in the vertebrates, one of the most striking features is the varied opinion on their origin and on the time of their earliest appearance in the embryo. For a long time, this has provided the basis for the much debated question of whether reproductive cells are segregated early or arise from epithelial cells as the result of cellular differentiation.

In lower vertebrates, so-called germ cells are described in the entoderm and mesoderm by various authors (Woods, '02; Wheeler, '99; Humphrey, '25, and others) both before and after the formation of the somites. These cells, which are described as reaching the genital anlage through various means, are usually larger than their immediate neighbors, and have a more distinct nucleus and a fairly definite boundary. The above characteristics and the fact that they can be distinguished from other surrounding cells at an early period lead some (Beard, '02; Allen, '09; Okkelberg, '21, and others) to think that they are early segregated cells. They have not, however, been traced, in most instances, into early cleavage stages, and thus no positive proof of an early separation and a definite germ tract has been offered.

Some of the other lower vertebrates provide well known instances of the formation of germ cells from the peritoneal epithelium. Bouin ('00) in his critical study of amphibia concludes that the reproductive cells have a mesenchymal and a peritoneal origin. In *Rana temporaria,* Gatenby ('16) describes the transition of peritoneal cells into germ cells. In the same manner, Lubosch ('03) accounts for the origin of the sex cells in *Petromyzon planeri.*

Literature provides us with practically no evidence of the presence of germ cells before the appearance of the gonad in higher vertebrates, especially in mammals. Their source here is said to be the peritoneal epithelium and in many cases the first generation of sex cells (Kingery, '17; Winiwarter and Sainmont, '08) is found to degenerate, the definitive cells being formed from the germinal epithelium (Allen, '23; Papanicolaou, '25, and others).

Wherever germ cells are found to be formed from epithelial cells it seems to support the increasing evidence in favor of the theory that sex cells may be differentiated from body cells and do not have a specific character.

It is with one of the cyclostomes, *Petromyzon marinus unicolor,* that the present investigation is principally concerned. The study [1] is made as an effort to ascertain whether germ cells are segregated early, or arise as the result of cellular differentiation.

Observations.

Large cells with round vesicular nuclei, which apparently are identical with those germ cells found by Okkelberg ('21) in the brook lamprey, have been observed by the writer in the caudal end of the embryo just before the appearance of the distinct entoderm and mesoderm. At this time the greater number of these cells lie immediately below the ectoderm ventro-lateral to the position of the future pronephric duct. Their distinct cell boundaries and characteristic roundness render them distinguishable from the irregular surrounding cells. Previous to this time, they are apparently similar in structure to other embryonic cells. As development proceeds and the mesoderm separates from the entoderm, these yolk laden cells are found in the mesoderm. Goette ('90) recognized similar cells (*Petromyzon fluviatilis*) occurring in the mesodermal plates directly ventrad and laterad of the pronephric duct. Large round masses of yolk, distinctly marked off from other entodermal cells just laterad to the myotomes, are considered by Wheeler in Petromyzon planeri to be reproductive cells. He finds that they become included in the mesoderm upon its lateral extension.

[1] The writer desires to express his appreciation for the helpful suggestions of Prof. H. D. Reed. To Prof. Gage he is, likewise, indebted for the loan of a complete series of early lake lampreys.

As the embryo of the lake lamprey becomes older, the number of these yolk-laden cells increases in the mesoderm as it separates from the entoderm caudally. Judging from the series at my disposal, there is apparently no particular arrangement of these cells at first, but with further differentiation of the mesoderm, they become grouped in bands, ventro-lateral to the pronephric ducts. Their characteristic large size and roundness make them easily recognizable among the neighboring cells.

At approximately the time that the larva breaks out of the egg membrane, the cells of the anterior levels reach a more medial position probably through a dorso-medial shifting of structures. In their new location they are now situated, for the most part, ventral to the pronephric ducts. Their roundness of form has been lost to some extent and they now possess a more flattened appearance, due, probably to the pressure of adjacent parts. In the caudal end of the embryo the bands of cells are still in the more lateral position and are widely separated.

In the cephalic region of a larva, older than the one described above, these large groups of cells are seen to practically come together medially. Often the nucleus with its vesicular appearance is hard to distinguish on account of the yolk-laden character of the cells. In the caudal direction, the large cells are still in a ventro-lateral position with respect to the pronephric duct. They retain a round form, in most instances, due to the more embryonic character of this part of the larva.

When the coelomic cavity forms, these yolk-laden cells become included in the somatic mesoderm. Their yolk granules, however, having begun to disappear, remain in a somewhat fragmented condition. As the yolk is gradually absorbed, the cell becomes smaller. By referring to Fig. 1, it will be seen that the chromatin granules in the large spherical nucleus appear large and stain deeply. One and sometimes two nucleoli are present. In the latter instance, one is usually larger and more intensely staining than the other. These large yolk-retaining cells, as Okkelberg claims in the brook lamprey, are always beneath the peritoneal epithelium from the time that it can be recognized as such.

At the same time that these yolk granules are being absorbed and are becoming less distinct, certain of the peritoneal cells ventral to the aorta and medial to the pronephric ducts begin to enlarge in situ. At this time the cells of the epithelium vary in shape, but most of them are of a flat elongated type. The first indication that some of these possess different potentialities is their increased size and a transition to a more spherical form. From this stage, they undergo a period of fairly rapid differentiation and acquire characteristics of germ cells found later in the definitive gonad.

The formation of germ cells from the peritoneal epithelium in the lamprey is not an entirely new observation. Lubosch ('03) believed that the peritoneal epithelium was the source of germ cells and follicle cells in *Petromyzon planeri*. He, however, did not give a detailed description of the process. On the other hand, since the germ cells were never seen in the peritoneal epithelium and because of their early history and structural characters in *Entosphenus wilderi*, Okkelberg concluded that the sex cells were not derivatives of the epithelium.

The process of the transition of peritoneal cells into germ cells can easily be found in larvæ which are 7–10 mm. in length. In these individuals, numerous cells of the peritoneal epithelium with deep staining nuclei are seen to be gradually changing from an oval to a spherical form (Fig. 3). The cell boundaries begin to appear more distinct. The cytoplasm is clearer as a whole, and seems to be proportionally larger in amount with respect to the nucleus. This is due to growth and also partly, no doubt, to a change in form.

As differentiation proceeds, the nucleus of the transforming cell becomes more vesicular and the chromatin is seen to clump, so to speak, at the intersections of the linen network (Fig. 2). Often two nucleoli are readily distinguishable. Figs. 4 and 7 show that during this time a change is also occurring in the cytoplasm. Besides increasing in amount, although slightly granular, it is becoming somewhat clearer.

In the course of further development, there is a corresponding change in the form of the immediately adjacent cells. They become flattened against the sex cells, presenting the appearance of crescent-shaped structures. From this stage they soon begin a growth up and over, so as to push the reproductive cells inward

(Fig. 6). Fig. 7 shows that some cells reach a greater size than others before being completely covered over by the growth of the adjacent peritoneal cells. In some cases such a large growth is attained that large bulgings are noticeable (Fig. 5).

As the cell is pushed under the epithelium, it enlarges slightly. The final result is a germ cell with moderately well defined cell boundary and a slightly granular cytoplasm. A definite nuclear membrane is noticeable. Oftentimes the reticulum is poorly defined and the deeply staining chromatin granules present a somewhat isolated appearance. A small lightly staining nucleolus and a larger more distinct one are usually present. In comparison to the final stage of the yolk-laden cells, they are identical in structure, and cannot be told from them. Both have reached a position immediately below the peritoneal epithelium.

Successive transitional stages provide evidence that germ cells arise from the peritoneal epithelium. Further support is furnished from the observation that germ cells attain a considerable size in the epithelium of which they are a part before being pushed inward.

This proliferation and formation of reproductive cells from the peritoneal epithelium is noticeable for a considerable time (7–15 mm. larvæ). The ability of the epithelium to transform into germ cells is gradually reduced, however, as the time is approached when a distinct gonad is recognizable.

Only a few enlarging peritoneal cells are found in 16 mm. larvæ. In some places, however, large germ cells are still found as a part of the epithelium. At this stage a thickening, indicating the region of the future gonad, is beginning to appear immediately below the dorsal aorta. The gonad is noticeable as a distinct structure in 18 mm. larvæ. This agrees with the conditions found in *Petromyzon planeri* by Lubosch. The germ cells found continuous with the epithelium at this time are not different structurally from those found in earlier stages.

The gonad appears as a single ventral median diverticulum in a 20 mm. larva (Fig. 9). For the most part, it is made up of germ cells and an epithelial covering, consisting of flat elongated cells with oval nuclei. Some of the germ cells still lie in the dorsal mesenchyme and have not migrated, or have not been included in the gonad. Epithelial cells are seen to be proliferated inward to furnish the bulk of the follicle cells. The follicular tissue soon isolates the germ cells, forming small follicles. In some cases, the large sex cells are very near the coelomic cavity, the only separation being the intervening cytoplasm of a flattened follicle cell. The structure of the gonad varies somewhat in its longitudinal extent. In some regions, it consists mainly of follicle and germ cells; at other levels, stroma is its chief constituent.

Later in a 26 mm. larva, after the migration of blood vessels and mesenchyme ventrally into the gonad, the sex gland is less dense than at 20 mm. At about this time, the germ cells begin a period of division and ordinary mitotic figures are occasionally encountered. The reproductive cells are arranged both in nests and as single elements. Where the latter condition occurs, the sex cell, in most cases, remains in a resting stage and has not undergone mitotic division. Nests, which are found less frequently, comprise a varying number of secondary germ elements. Already, in some places, the mass of germ cells is being broken up by the continual ingrowth of follicle cells. The gonad is a narrow diverticulum in some regions, being little wider than a single germ cell. At other levels, no reproductive cells are present, and the sex gland consists of a loose stroma and a cuboidal epithelial covering. For the most part, the majority of the germ cells are in a resting condition, though mitotic stages and completely divided cells are found.

The sex gland, meanwhile, is extending in a cephalic and caudal direction. This leads to the question of whether the germ cells of these extremities arise as a result of division of resting cells or are formed anew from the epithelium of the growth areas. In the larvæ which I have studied, evidence indicates that the former occurs in most instances. Numerous mitoses are found in the peritoneal epithelium but there is little evidence of a great transition of epithelial cells of these regions into reproductive cells.

In 30–33 mm. larvæ, the gonad extends a greater part of the length of the coelom. In Fig. 8 it is also seen to be considerably larger than in the 20 mm. larva of Fig. 9. Some of the germ cells are still in the resting condition, but, apparently, the greater amount of division occurs at this time, from the frequent mitotic figures encountered. Many nests with various numbers of secondary sex elements are present. The reproductive cells, for the greater part, are peripherally situated in the gonad, being directly under the epithelium. The central part of the sex gland consists mainly of loose stroma and blood vessels. No maturation phenomena are observed in larvæ of this stage.

In the sex gland of a 50 mm. larva, the nests of germ cells are arranged around a medulla of connective tissue which is continuous dorsally with that of the mesentery. Evidently the nest is differentiated as a whole. Some nests contain, for the most part, cells in a resting condition, while in others various mitotic figures are found. In very large nests indications of degeneration are found. Since this appears in only the large ones, the insufficient blood supply and crowding are probably the causes. It is impossible to say whether all the cells of an individual nest result from the mitosis of an original single germ cell, since the migration of follicle tissue inward causes a breaking up and a continual rearrangement. At this time, the sex cells are apparently of an indifferent character, and the sex of the individual could not be established with certainty. Beyond this stage, a detailed study was not made. The maturation stages and the various factors that may be influential in deciding sex were not investigated.

Very few large cells are found to originate from the epithelium after the formation of the gonad. In a 33 mm. larva a few peritoneal cells are found enlarging and figure 8 shows a large reproductive cell continuous with the epithelium of the sex gland. Since the proliferation is usually not great in larvæ as old as this, this is regarded as an unusual occurrence, depending in all probability upon the environmental changes, and various conditions existing within the gonad. Of course, there is the possibility that germ cells might arise from follicle cells or be proliferated inside the gonad before a great growth is attained. In all the material examined, however, there is no indication of such a phenomenon. If there is much formation from the peritoneum after a distinct gonad is recognizable, one would expect to find large and enlarging cells in the epithelium. The increased number of reproductive cells after the appearance of the gonad results, mainly, I believe from mitotic division of germ cells already differentiated.

Discussion.

In this study of the origin of the germ cells, the first question that arises is the one concerning the fate of the yolk-retaining cells, distinguished as primordial germ cells by some authors. Apparently from the previous investigations on vertebrates, they have two alternatives; either they persist to form the definitive germ cells of the adult, or they degenerate and disappear, the definitive sex cells then arising from peritoneal cells. Just how extensive this degeneration is seems to vary in the different vertebrates studied. Bouin holds that most of the primordial germ cells degenerate in *Rana temporaria* and the definitive sex cells have both a mesenchymal and a peritoneal origin. According to Dustin ('07), in Amphibia (*Triton alpestris, Bufo vulgaris* and *Rana fusca*) some of the primordial germ cells which do not degenerate form functional cells in the gonad. He states, however, that a second generation from the epithelial covering of the gonad furnishes the greater portion of the definitive sex cells. Okkelberg believes that the primordial germ cells are the sole source of the definitive cells in the brook lamprey, and none are formed from the epithelium.

In Petromyzon marinus unicolor, most of the large yolk-laden cells, which for the sake of clearness may be termed primordial germ cells, after losing their yolk are indistinguishable from those sex cells having a peritoneal origin. From their position many of them would naturally become included in the gonad. My material shows that some may degenerate, and evidently the rest persist and finally become definitive germ cells. Most of the definitive sex cells, however, probably originate from peritoneal cells. Of course, there is the possibility that these epithelial cells which differentiate into germ cells were the primordial germ elements that reached this position through shifting of structures. That this is not the case, is evidenced by the fact that ordinary epithelial cells indistinguishable from others of the peritoneum are seen to transform into germ cells. It is, likewise, illogical to conceive of the primordial germ cells dedifferentiating into cells indistinguishable from other peritoneal cells and then again gradually take the characteristics of sex cells. To believe that these peritoneal cells are not somatic cells, but cells which have maintained their embryonic structure and have not specialized in a particular direction would seem even more unreasonable.

If germ cells do not have an epithelial origin, there is no way of accounting for the varying sizes found, since it is highly improbable that the so-called primordial germ cells would exhibit such a variation in size and such a close relation and resemblance to the peritoneal cells. Although large germ cells may appear to be continuous with and actually a part of the epithelium, this does not necessarily justify, in my mind, the conclusion that they have a peritoneal origin. To be certain that germ cells are derived from the epithelium, it is necessary for one to identify successive transitional stages.

Another important question is the significance of the primordial germ cells. Structurally and morphologically at one time, they are indistinguishable from other embryonic cells in the lamprey. Since they cannot be traced back to cleavage stages, there seems to be no logical justification for a belief in a distinct line of germ cells which differ physiologically from other embryonic cells. If, then, there is apparently no basis for the evidence of an early germ tract, the question naturally arises why some cells differ from other embryonic cells in having a yolk-retaining property for so long a time.

Two suggestions are given in the following pages as possible explanations for the presence of these so-called primordial germ cells. In the first place, it is evident that, at one time, the primordial germ cells are in the same position as the cells which later become part of the peritoneal epithelium, but as the latter are organized, the yolk-retaining cells take their position below the peritoneum. If this is the case, the process of germ cell formation is no different from that in later stages (transition of peritoneal epithelial cells into germ cells). The whole thing represents, then, a continuous process of germ cell formation as a result of cellular differentiation from a very early stage up until, and after in some particular cases, the formation of the gonad. If one accepts this analysis, the fundamental property of growth and differentiation becomes the explanation of germ cell formation. No doubt, the reason for degeneration, in some instances, would be the environmental factors, insufficient vascular supply, and unfavorable conditions existing within the body of the individual.

The second suggestion is one from a phylogenetic point of view. In some arthropods, where all the egg and larval material is laid out for the formation of definite areas in the adult, germ cells may be specially provided with a large amount of nutrient material (Gatenby '24). How this extra supply of food material acts is not known, but as Gatenby intimates it may contain enzymes or other substances which suppress the differentiation of the germ cells during the embryo formation, and keeps them isolated. This, no doubt, prevents their passage into the stream of differentiating somatic cells, whose influence might result in the loss of germ-cell integrity. In mammals, on the other hand, where evidence is increasing that a somatic cell may become a germ cell under the proper stimulus, and where no special provision is made, as in insects, there is found no proof of segregation in cleavage stages. In these higher vertebrates, the cell nucleus has apparently unlimited power and germ cell formation is the result of growth and differentiation.

In the lamprey, it may be that these large yolk-retaining cells are the remnants of the conditions found in lower forms. The germ cells which are formed from peritoneal cells represent a step in that evolutionary process which reaches its height in the mammals.

Even a third interpretation might be presented, namely, that the primordial germ cells may act as a stimulating factor to the formation of germ cells from the epithelium and a gonad. They are found in many vertebrates, yet positive proof that they give rise to all of the definitive sex cells is lacking.

Further comment does not seem necessary. It is evident that the definitive germ cells have, for the most part, a peritoneal origin in the lake lamprey, yet there are primordial germ cells, the presence of which is perplexing.

Conclusions.

1. The definitive sex cells of the lake lamprey originate from the so-called primordial germ cells and also from peritoneal cells.

2. The primordial germ cells are first distinguishable as large

yolk-laden cells in the caudal end of the embryo just before the appearance of a distinct mesoderm and entoderm.

3. The finding of the successive stages from the ordinary epithelial cell to the definitive germ cell offers evidence that germ cells are formed from the peritoneal cells.

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EXPLANATION OF FIGURES.

FIG. 1. Transection of a 6.5 mm. larva, showing primordial germ cell in which the yolk is disappearing. × 380.

FIG. 2. Transection of a 9 mm. larva, showing the second stage in the transition of a peritoneal cell into a germ cell. × 640.

FIG. 3. 9 mm. larva, showing the first stage in the enlargement of a peritoneal cell. × 640.

FIG. 4. Enlargement of a definitive sex cell in the peritoneal epithelium of a 7 mm. larva. × 380.

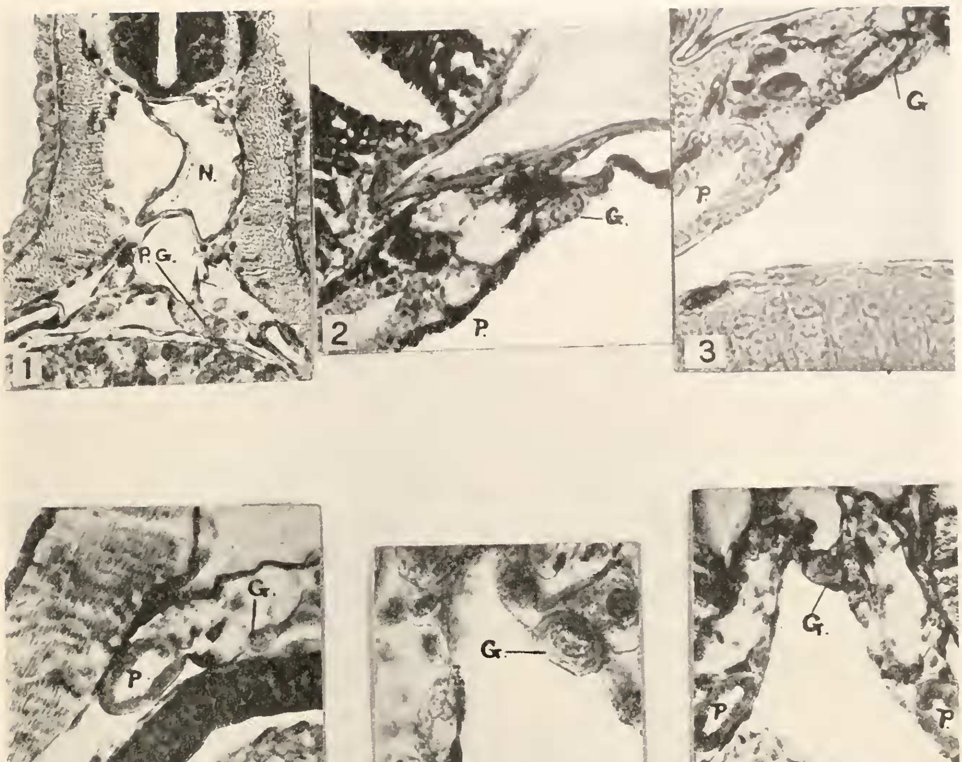
FIG. 5. This illustrates the large size that some germ cells reach before being proliferated. Section of a 10 mm. larva. × 790.

FIG. 6. Section through a 10 mm. larva. The adjacent epithelial cells are growing up around the germ cell. × 380.

FIG. 7. 9 mm. larva. Enlarged germ cell is shown in the epithelium. × 790.

FIG. 8. Gonad of a 33 mm. larva. Germ cell is continuous with the epithelium. × 380.

FIG. 9. Section through gonad of a 20 mm. larva. × 380.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